SYNOPSIS

Of

Ph.D Research Work

Entitled

"Studies on the Assessment of Microplastic in the Soil of Some Agricultural Areas of Subarnrekha Origin and its Impact on Earthworm (*E. fetida*) Species"

To be undertaken by

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TITLE

"Studies on the Assessment of Microplastic in the Soil of Some Agricultural Areas of Subarnrekha Origin and its Impact on Earthworm (*E. fetida*) Species"

STATE OF THE ART

Microplastics (MPs) represent an alarming and emerging threat in our contemporary era, posing profound environmental and health risks. MPs are referred to as tiny plastics that are below 5mm in size (Hebner & Maurer-Jones, 2020). They fall into two main categories: primary microplastics, intentionally manufactured small plastic particles, and secondary microplastics, which result from the fragmentation of larger plastic materials through various processes and degradation (Napper et al., 2015, Smith et al. 2018). Recent publications have documented the MPs pollution in water, soil, plants, and air (Koelmans et al., 2019, Qiu et al. 2022, Amato-Lourenço et al. 2020, Azeem et al., 2021).

The pervasive presence of microplastics in the food chain has become a growing concern, as these minute plastic particles have been identified in various organisms across different ecosystems at different trophic levels (Jin et al., 2021).

Research indicates that microplastics tend to bioaccumulate within different trophic levels, posing a potential threat to various organisms (O'Connor et al., 2022). However, there is currently no substantiated evidence supporting the idea that microplastics undergo biomagnification. In other words, the likelihood of microplastics biomagnifying appears to be low (Akhbarizadeh et al. 2019, Miller et al. 2020).

Beyond Aquatic ecosystems, MPs have also been detected in terrestrial environments including. Soil-dwelling organisms, such as earthworms and insects, can ingest MPs, leading to potential contamination of the terrestrial food chain (Qiu et al., 2022, Zhang et al. 2022, Dissanayake et al., 2022).

In my work, the primary focus will be on earthworms and soil fertility. The earthworms, often regarded as unsung heroes of the soil, play a pivotal role in maintaining soil health and fertility (Van Groenigen et al., 2014). Their burrowing activities enhance soil aeration, water infiltration, and nutrient cycling, making them vital components of terrestrial ecosystems that further

contribute to the sustainability and environmental friendliness of organic agriculture (Cao, J., et.al. 2015).

MPs that accumulate in the soil, and come into direct contact with earthworms, potentially leading to myriad repercussions (Qiu et al. 2022). Studies have revealed the detrimental impact of MPs on the growth and reproduction of earthworms (Gong et al., 2023). Various investigations revealed that the exposure to microplastics can inhibit the growth of earthworms, potentially affecting their overall fitness and population dynamics (Gong et al., 2023). Additionally, it was observed that microplastics can disrupt reproductive processes in earthworms, raising concerns about their long-term sustainability within contaminated environments. A decrease in cocoon production has also been observed due to exposure to MPs (Y. Chen et al. (2020)).

The activities of earthworms, which are vital for soil health and nutrient cycling, can be influenced by microplastic pollution. Lwanga et al., (2017) have documented the changes in the earthworm behavior when exposed to microplastics, including alterations in burrowing patterns and foraging activities. Such behavioral modifications could have cascading effects on soil structure and ecosystem processes (Lwanga et al., 2017, Zhong et al., 2022).

Rodríguez-Seijo et al. (2017) have highlighted that microplastic exposure has a severe/ adverse effect on the immune systems of earthworms. Their research suggests that microplastics can compromise the immune defenses of earthworms, potentially rendering them more susceptible to environmental stressors and pathogens, further jeopardizing their survival.

Microplastics serve as carriers for various other pollutants, such as heavy metals, persistent organic pollutants (POPs), and hydrophobic organic pollutants (HOCs) (Huang et al., 2021). Beyond direct effects on earthworms, the presence of microplastics in soil ecosystems may indirectly influence the bioavailability of other soil contaminants. Huang et al. (2021) suggested that microplastics could alter the distribution and transport of other pollutants, potentially exacerbating the overall environmental risks associated with soil pollution.

MPs exposure exhibited significant histopathological damage to the digestive tissue and the intestinal tissue of the earthworm (Y. Chen et al., 2020b, Rodríguez-Seijo et al. 2017).

Several studies showcased the detrimental effects of polystyrene MPs on earthworms, resulting in oxidative stress marked by the alterations in the activities of key enzymes like glutathione,

superoxide dismutase, and acetylcholinesterase (Jiang et al., 2022, Y. Chen et al., 2020b).

PROBLEMS TO BE INVESTIGATED

Presence and Distribution of Microplastics: Studies have shown the contamination of MPs in the agricultural soil of several states in India, such as Bhopal, due to increased usage of plastic products in agriculture. However, there is a lack of information regarding the distribution of microplastics in the **agricultural soil** near the Subarnarekha origin poin**t** Rani Chuwan

Nutrient profile of soil with and without the contamination of microplastics

Physiological Effects on Earthworms: The physiological consequences such as Growth, cocoon formation, SOD, GSH, AChE of earthworms ingesting or being exposed to microplastics remain largely unexplored in the context of Ranchi. Understanding how these particles affect earthworm health and functions is vital and influence if any in soil profile

Addressing these aspects of the problem will provide valuable insights into the potential threats microplastics pose to earthworms, and soil health in Ranchi, Jharkhand.

OBJECTIVES

1. Assessment of Microplastics in Soil from various agricultural locations near the Subarnarekha origin point

- To determine the extent and distribution of microplastics in the soil near the agricultural field surrounding the Subarnrekha Origin point(Rani Chuwan). Because it is a crucial water source for farming fields in the region. Given that the river is susceptible to plastic pollution, the inflow of microplastics into the water is a significant concern. These microplastics have the potential to accumulate in the soil of the adjacent fields, raising environmental and agricultural implications.

Nutrient Profile Analysis of the soil with the help of soil testing KIT

Nutrient Profile Analysis

Soil nutritional value is a crucial determinant of soil fertility (Chaudhry et al., 2024). It refers to the availability of the essential nutrients (such as Nitrogen, Phosphorus, Calcium etc.) present in the soil. To investigate the impact of microplastic contamination on soil fertility, the nutritional value of the soil sample under different conditions is compared. Soil samples collected from the agricultural area of Ranchi are divided into three parts A, B and C. A is served as a control group without any manipulation, in part B the microplastics will be removed from the soil using an established extraction method, and in part C a specified amount of the microplastics (eg.100 g) will be introduced. Earthworms will be cultured in each part of the soil sample. After 30 days of exposure, histochemical and enzymatic assay analysis will be performed to evaluate the impact of microplastic contamination on earthworms. Nutritional values of soil samples among different conditions (part A, part B and part C) were evaluated and compared to determine the potential impact of microplastic contamination on earthworms and soil fertility.

2. Assessment of Microplastics in Various Organs of Earthworms:

- To investigate the presence and accumulation of microplastics within the various organs (e.g., Different regions of gut, Reproductive organs, Nephridia, Skin etc.) of earthworms residing in the study area.

3. Studying Enzyme Assays for Stress Assessment:

- To assess enzyme activity levels, including Superoxide Dismutase (SOD), Acetylcholinesterase, and Glutathione (GSH) content in earthworms as indicators of oxidative stress, neurotoxicity, and metabolic alterations resulting from microplastic

exposure.

- To correlate the results of SOD, Acetylcholinesterase, and GSH assays with the extent of microplastic exposure, allowing for the identification of stress thresholds and elucidating the mechanistic pathways through which microplastics may impact earthworm physiology.
- Correlation between earthworm health and soil nutrient profile

HYPOTHESIS

- Microplastics are expected to be present in the agricultural soil present at the origin of Subarnrekha River
- Earthworms exposed to microplastics in soil may exhibit higher accumulation levels of these particles in their tissues (eg. Different regions of the gut, Reproductive system, Nephridia, Skin etc.)
- It is expected that Microplastic exposure may create oxidative stress and neuronal toxicity in earthworms.

METHODOLOGY

1. Detection of MPs in the soil sample

- **Gathering soil samples from the study area-** 8 locations of agricultural fields around the origin of Subarnarekha River are selected (**N**-23°18'07"N 85°11'03"E, **E**-23°18'06"N 85°11'03"E, **S**-23°18'06"N 85°11'03"E, **W**-23°18'06"N 85°11'02"E, **NE**-23°18'06"N 85°11'03"E, **SE**-23°18'06"N 85°11'03"E, **SW**-23°18'06"N 85°11'02"E, **NW**-23°18'06"N 85°11'02"E). Soil to a depth of 0-10 cm and 10-20 cm is dug out from each location and 1 kg sample collected in a stainless steel container and labeled.

- **Filtration of microplastics from soil specimens-**

  ➔ The specimen will be air-dried and sieved through 2 mm mesh to remove the large

debris.

- ➔ Density separation will be done by adding high-density NaCl solution (density ~1.2 g/cm³) and decanting the floating MPs supernatant into a clean container.
- ➔ The supernatant will be filtered through a cellulose filter (0.45µm)

● **Identification of microplastics in the soil sample**

- ➔ Visual examination of particles will be done under a stereomicroscope and data (number, shape) will be recorded
- ➔ Chemical identification will be done via FTIR(Fourier Transform Infrared Spectroscopy)

2. Detection of MPs in earthworm and histopathological studies

● **Sample collection-**

- ➔ *E. fetida* will be collected and acclimatized to the environment.

● **Soil preparation-** Three types of soil is prepared for the experiment

- ➔ Part A- Soil collected from the site
- ➔ Part B- Microplastic free soil
- ➔ Part C- Microplastic added soil (10g/Kg, 50g/Kg, 100g/Kg)

● **Microcosm setup-** 5 setups are prepared using sizes 30cm X 20cm X 10cm containers. Three replicates of each treatment are set up.The Microcosm environment will be maintained and the experiment will run for 30 days.

● **Detection of MPs using histochemical techniques.**

- ➔ Earthworms from each microcosm will collected and rinsed after 30 days.
- ➔ 10 % KOH solution will be used for the digestion of the earthworm tissues (digestive, intestinal tissues, reproductive organs) in 1:3 ratio.
- ➔ The digestate is filtered using a cellulose filter and identified via visual (stereomicroscope) and chemical (FTIR) method

3. Studying Enzyme Assays for Stress Assessment.

- Investigate the impact of controlled microplastic exposure on earthworms by quantifying changes in key enzymatic activities associated with stress responses (SOD, Acetylcholinesterase, and GSH).
- Establish a correlation between the concentrations of microplastics to which the earthworms are exposed and the corresponding changes in enzyme activity levels.

STATISTICAL ANALYSIS

Statistical methods (ANOVA) will be employed to analyze the data collected from laboratory experiments and field surveys. These analyses will help establish relationships between microplastic pollution and observed effects on earthworms and soil ecosystems.

EQUIPMENT REQUIRED

Microplastic Isolation and Characterization:

1. Soil collection tools (shovel, trowel, etc.)
2. Microplastics of various polymers as required by the study
3. Sieves with mesh sizes of 5 mm, 1 mm, and 500 µm
4. $ZnCl_2$ solution for density separation
5. Nile red
6. Spectrophotometer, FTIR
7. Optical microscope
8. Glassware and laboratory supplies

Histopathological Examination:

1. Formalin (for tissue preservation)
2. Histological dyes and staining equipment
3. Microtome
4. Microscope and microscope slides
5. Recording and data analysis tools

Enzyme Assays for Stress Assessment:

1. Laboratory equipment for tissue homogenization
2. Spectrophotometer
3. Reagents for Superoxide Dismutase (SOD) activity assay
4. Reagents for Glutathione (GSH) level measurement
5. Reagents for Acetylcholinesterase (AChE) activity assay

SIGNIFICANCE OF STUDY

This study endeavors to provide crucial insights into the ecological dynamics of agricultural landscapes in Jharkhand by examining the presence or absence of microplastics (MPs) in the soil and investigating their impacts on the resident earthworm population. In the absence of microplastics, earthworms may not be exposed to the associated risks linked to the ingestion or interaction with these particles. This could contribute to a healthier soil environment for earthworms, promoting their natural behaviors without the potential adverse effects associated with microplastic contamination. Through this research, we aim to enhance our understanding of the intricate interactions between microplastics and earthworms in the context of Jharkhand's agricultural environments, contributing valuable knowledge for informed environmental conservation practices and sustainable land management.

TO THE PRESENT-DAY PROBLEM AND NEED OF THE FUTURE

WORK

This research explores the presence of microplastics in Jharkhand's agricultural soil and their impact on organisms, focusing on earthworms. The findings will guide future studies on various soil biota and plants. As the annual escalation in plastic overuse, coupled with inadequate management practices, is anticipated to fuel a continued rise in microplastic contamination in the future the study emphasizes the urgency of addressing microplastic pollution, serving as a call to action for policymakers, communities, and industries.

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